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Pharmacopoeial and *In-silico* studies of Unani Joshanda: A potent drug for management of infectious diseases

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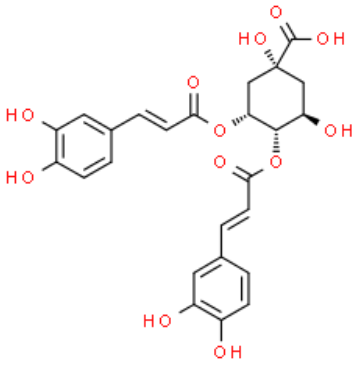
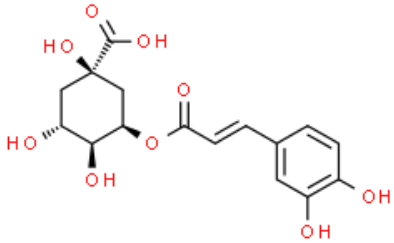
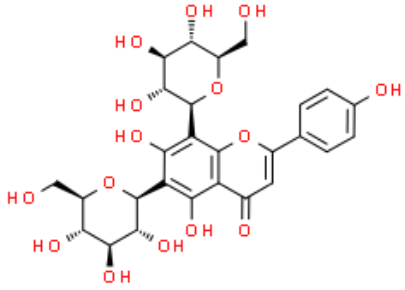
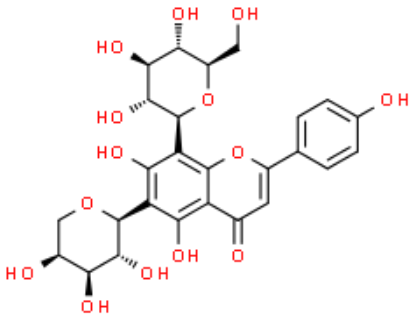
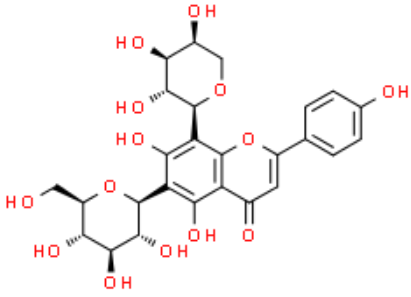
Abstract
Unani system of medicine is one of the most well-known traditional system of medicine. It provides promotive, preventive, curative, rehabilitative, safe health care with holistic approach.In Unani medicine; various general measures are mentioned to prevent epidemic, endemic and pandemic diseases. Unani Joshanda is a formulated drug released in the Guidelines for Unani Practitioners in management of COVID 19 by Central Council for Research in Unani Medicine (CCRUM) after several literature surveys. The drug comprises of three fruits as ingredients namely Behidana (*Cydonia oblonga*), Unnab (*Zizyphusjujuba*) and Sapistan (*Cordia myxa*). The combined action of these fruits enhance and endorse the human immune system and plays a key role in management of SARS like infections. The aim of present study is to evaluate quality standards using pharmacopoeial methods and efficacy through *in-silico* studies for Unani Joshanda drug.

Keywords: *in-silico* studies, Unani Joshanda, Behidana, Unnab, Sapistan, Covid 19, SARS

1. Introduction
Since theWorld Health Organisation has declared the COVID 19 as a pandemic and global health emergency, several pharmaceutical industries are engaged in the development of newer drugs to combat the coronavirus and its extreme speedy transmission. Unani system of medicine is one of the most well known traditional system of medicine. It provides promotive, preventive, curative, rehabilitative, safe health care with holistic approach [1]. In Unani medicine, various general measures are mentioned to prevent epidemic, endemic and pandemic diseases. The classical text of Unani medicine has recommended many formulations to be taken during such situations [2]. As per Unanischolarswisdom, improving immunity with immune boosters is one of the key approach to maintain the good health andto prevent many diseases. Unanischolars have prescribed several single and compound formulations which improves the host immunity during the outbreaks. Popularly many fruits, vegetables, vitamins, minerals, antioxidants, probiotics, functional foods and others complementary and alternative medicines acts as immuneboosters that strengthens the immune system of our body [3].Unani Joshanda is one such formulated drug released in the Guidelines for Unani Practitioners for COVD 19 by Central Council for Research in Unani Medicine (CCRUM) after several literature survey. The drug comprised of three fruits as ingredients namely Behidana (*Cydonia oblonga* Mill.), Unnab (*Zizyphus jujuba*) and Sapistan (*Cordia myxa*). The combined action of these fruits enhance and endorse the human immune system and plays a key role in management of SARS like infections. The detailed description of the individual ingredients are as follows:

1.1 Behidana
It is botanically known as *Cydonia oblonga* Mill. and belongs to the family Rosaceae. Other common names are Quince, Behi, Bahee Dana, Strythion and Safarjal [4]. Medicinally useful parts of this plant are Fruit and seeds; Fruit - round to peer like, fragrant and consists of yellow flesh with many seeds. The seeds consists of organic acids such as citric, ascorbic, malic, quinic, shikimic, fumaric acids, 3-O-caffeoylquinic, 4-O-caffeoylquinic, 5-O-caffeoylquinic, 3,4-dicaffeoylquinic acids, lucenin-2, vicenin-2, stellarin-2, isoschaftoside, schaftoside, 6-C-pentosyl-8-C-glucosyl chrysoeriol, 6-C-glucosyl-8-C-pentosyl chrysoeriol etc., [5]. The Molecular docking analysis of binding domain of SARS-CoV-2 Spike (S) glycoprotein and Main Protease of some chemical constituents present in Behidana are shown in Table 1.

Table 1: Binding Energies of some chemical constituents present in Behidana with SARS-CoV-2 spike (S) glycoprotein and SARS-CoV-2 main protease (Mpro)

S. No.	Chemical Constituents	Compound Structure	Molecular docking analysis binding domain of SARS-CoV-2 Spike (S) glycoprotein (6LZG) and Main Protease (7BQY)	
			SARS-CoV-2 spike (S) glycoprotein Binding Energy (Kcal/mol)	SARS-CoV-2 main protease (M^{pro}) Binding Energy (Kcal/mol)
1	3,4-Dicaffeoylquinic acid		-6.6 (R403, R408, Q493, G496)	-8.7 (F140, L141, S144, H163, R188, T190)
2	5-O-Caffeoylquinic acid		-7.0 (Y453, Y505)	-7.3 (T190, N142, S144, C145, H163, Q189)
3	Vicenin-2		-6.7 (R403, Y505)	-8.2 (Y54, N142, G143, S144, C145, P168)
4	Isoschaftoside		-6.7 (Y453, Q493, S494, E406)	-8.9 (L141, R188, T190)
5	Schaftoside		-6.9 (Y453, G496, Q493)	-8.3 (N142, S144, E166, L167)

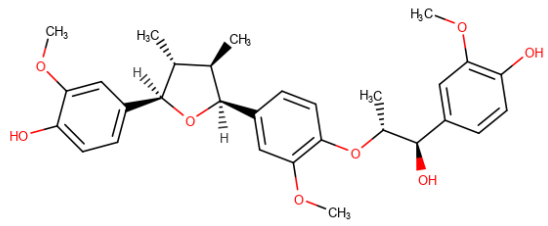
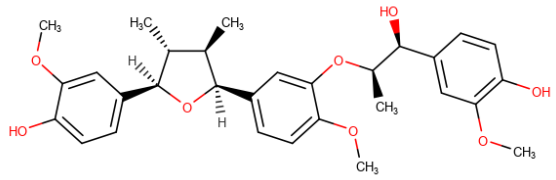
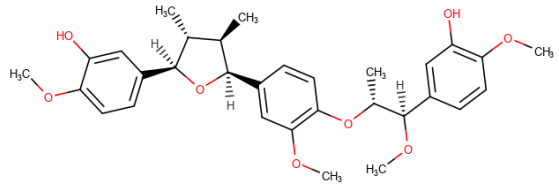
Therapeutically the seeds acts as astringent, emollient and are used to treat various ailments viz. diarrhea, dysentery, cough, sore throat, excess sneezing, fever, rhinitis, bronchitis, tuberculosis, intestinal colic and constipation [4-6].

1.2 Unnab

The Unnab is botanically called as *Ziziphus jujube* Mill. With Synonyms: *Z. Mauritiana* Lam, *Z.sativa* Gaertn. It belongs to the family Rhamnaceae and commonly known as Jujube,

Chinese date or red date[7]. The medicinal properties of this plant is found mainly in fruits which are drupes or berries and are red in colour with sweet taste. The main chemical constituents of the drug are vitamin C, B1 (thiamine) and B2 (riboflavin). It also contain high level of vitamin P (bioflavonoid), pectin A, 2,3, 6-tri-o-acetylD lactose units, rich in fatty acids and Zijusesquilignan A, Zijusesquilignan B and Zijusesquilignan C. [8-9]. The Molecular docking analysis of binding domain of SARS-CoV-2 Spike (S) glycoprotein and Main Protease of some chemical constituents present in Unnab are shown in Table 2.

Table 2: Binding Energies of some chemical constituents present in Unnab with SARS-CoV-2 spike (S) glycoprotein and SARS-CoV-2 main protease (Mpro)

S. No.	Chemical Constituents	Compound Structure	Molecular docking analysis binding domain of SARS-CoV-2 Spike (S) glycoprotein (6LZG) and Main Protease (7BQY)	
			SARS-CoV-2 spike (S) glycoprotein Binding Energy (Kcal/mol)	SARS-CoV-2 main protease (M^{pro}) Binding Energy (Kcal/mol)
1.	Zijusesquilignan A		-7.2 (R403, D405, R408)	-7.8 (T24, T26, N119, N142)
2.	Zijusesquilignan B		-7.2 (Y453, G496)	-6.5 (T190)
3.	Zijusesquilignan C		-6.9 (Q409, K417, Q493, N501)	-7.8 (T45, S46, M165)

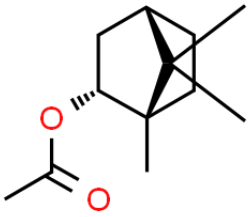
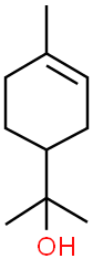
It stimulates bile production, promote circulation, lowers plasma cholesterol, prevent allergies and possess antidiarrhoeal properties [8, 10, 11].

1.3 Sapistan

The drug sapistan is scientifically known as *Cordia myxa* L. With Synonyms: *Bourreria glabra* G. Don, *C. Ixiocarpa* F. Muell, *C. latifolia* Wall. ex G. Don, *C. myxa*var. *Ixiocarpa* (F.Muell) Domin, *C. officinalis* Lam., *C. paniculata* Roth, *C. petta-pelioporet* B. Heyneex Roth, *C. Scabrifolia* Benth. Ex Griseb, *C. Sebestena* Forssk, *Ehretia glabra* Roth ex Roem & Schult, *Gerascanthus myxus* (L.) Borhidi [12]. It belongs to the family Boraginaceae. Other common names are Indian cherry, Lasora, sebestanplum [12]. Medicinally active part of the plant is fruit which ismucilage and sweet in taste.

The Chemical Constituents found in the fruit are Bornyl acetate, α-terpineol, fibres, proteins, vitamins and carbohydrates [13]. The drug acts as diuretic, analgesic, anti-inflammatory, immunomodulatory and antimicrobial drug and used to treat respiratory infections, cough, sore throat, gastrointestinal disorders [13]. The Molecular docking analysis of binding domain of SARS-CoV-2 Spike (S) glycoprotein and Main Protease of some chemical constituents present in Sapistan are shown in Table 3.

Table 3: Binding Energies of some chemical constituents present in Sapistan with SARS-CoV-2 spike (S) glycoprotein and SARS-CoV-2 main protease (Mpro)

S. No.	Chemical Constituents	Compound Structure	Molecular docking analysis binding domain of SARS-CoV-2 Spike (S) glycoprotein (6LZG) and Main Protease (7BQY)	
			SARS-CoV-2 spike (S) glycoprotein Binding Energy (Kcal/mol)	SARS-CoV-2 main protease (M^{pro}) Binding Energy (Kcal/mol)
1.	Bornyl acetate		-4.6 (G496)	-4.9 (C145)
2.	α-terpineol		-5.0	-4.8 (H164)

From the *in-silico* studies, it has been observed that the binding energy of phyto-constitutents present in the Unani Joshanda shows negative binding energy with S protein and main protease of SARS-CoV-2. The negative binding energy indicates that the phyto-constituents have the potential to inhibit the S protein and M^{pro} of SAR-CoV-2 and acts as immunity booster. Since more number of herbal productsin the name of Immune boosters are arriving in the market day by day the quality assurance of such intakes is an important factor and needs to be standardised and evaluate the efficacy before marketed [14]. Hence, the aim of the study is to evaluate the Unani Joshanda drug with precise scientific parameters and *in-silico* studies to lay out the identity, purity, quality and efficacy of the drug.

2. Materials and Methods

2.1 Formulation Composition of Unani Joshanda

Unani Joshanda is the powdered formulation made with the ingredients given in Table 4. The ingredients were collected from Chennai local market.

Table 4: Formulation Composition of Unani Joshanda

1.	BehidanaUPI–II	*Cydonia oblonga* Mill.	Seeds	3g.	30g.
2.	Unnab UPI-VI	*Zizyphusjujuba*Lam.	Fruit pulp	5 Nos.	50 Nos.
3.	Sapistan API-VI	*Cordia myxa* L.	Fruit	9 Nos.	90 Nos.

2.2 Method of Preparation

All the ingredients were taken of pharmacopoeial quality. The ingredients were cleaned, dried, powdered and sieved through 40 mesh. The sufficient quantity of powdered ingredients were mixed in a wide panwithout lumps and stored t in an air tight container for further studies.

2.3 Pharmacognostical Studies

The pharmacognostical studies such as Powder microscopy was carried out using standard method [15]. The 5g of sample was weighed and mixed with 50 ml of water in a beaker with gentle warming till completely dispersed in water. The mixture was then centrifugedand supernatant was decanted. The sediment was washed several times with distilled water, again centrifuged and decanted. Then few mg of the sediment was taken and mounted in glycerine, phloroglucinol and concentrated hydrochloric acid separately to locatelignified cells.

2.4 Physicochemical parameters

The powdered Unani Joshanda was used for quantitative determination of physicochemical parameters likeloss on drying, total ash, acid-insoluble ash, pH, volatile oil content and extractive values were determined [15].

2.5 Development of HPTLC Finger print profile

2.0 gm of sample was extractedwith 20 ml of alcohol and chloroform separately and refluxed on a water bath for 30 min. The extract was thenfiltered and concentratedto 5 ml to carry out the thin layer chromatography. The HPTLC studies were performed on aluminium plates pre-coated with silica gel 60F254 (Merck, Germany). The extracts were applied on the plate of 10 x 10 cm by HPTLC as bands of 8 mm of each with help of CAMAG ATS4 sample applicator. The plates were developed in a CAMAG twin-trough chamber equilibrated with a mobile phase for 30 mins.The solvent system used to develop HPTLC finger print profilefor alcohol extract is Toluene: Ethyl acetate: Formic acid (8.2: 1.8: 0.4); 15µl) and for Chloroform extract is Toluene: Ethyl acetate: Formic acid(8.2: 1.8: 0.4); 10 µl). The plates were developed upto 8 cm, air dried and scanned at wavelength of 254 and 366 nm using CAMAG TLC Scanner. After recording the chromatograms, the plates were derivatized with Vanillin Sulphuric acid and heated at 105ºC till the development of colour of bands and observed under UV and white light [16-17].

2.6 Quality Control Parameters

2.6.1 Microbial load

The evaluation of microbiological contamination viz total bacterial count, total yeast count and specific pathogens like *Escherichia coli, Salmonella species, Pseudomonas aeruginosa* and *Staphylococcus aureus*were performed as per the approved WHO guidelines [18-19].

2.6.2 Heavy Metal Detection

Heavy metals analysis was performed using the instrument atomic absorption spectrophotometer (AAS) for analysis of heavy metals such as lead, cadmium, arsenic and mercury. The Guidelines used for the study [20].

2.6.3 Aflatoxin detection

The aflatoxin detection was carried out using the instrument VICAM Afla Test Fluorometer by following VICAM Instruction Manual (2014). The Pretreated sample was applied to the Afla test column bound with specific antibodies to aflatoxin. The column was then washed with methanol and measured in a fluorometer. The results were interpreted after validation check carried out using TLC [18, 21].

3. Results and discussion

3.1 Microscopy

Epidermal cells with mucilage hairs; pigment layer in surface view; endosperm cells in surface view with aleurone grains and oil globule (Behidana); Thin walled round to oval parenchyma cells from the mesocarp filled with reddish brown contents; polygonal slightly thick walled reddish brown epidermal cells in surface view; druses of calcium oxalate crystals upto 30µ (Unnab); Thick walled larger epidermal cells in surface view; stone cells of two different types, lignified thick walled with broad lumen and narrow lumen upto 250µ; unicellular covering trichomesupto 200µ (Sapistan) were shown in Figure 1.

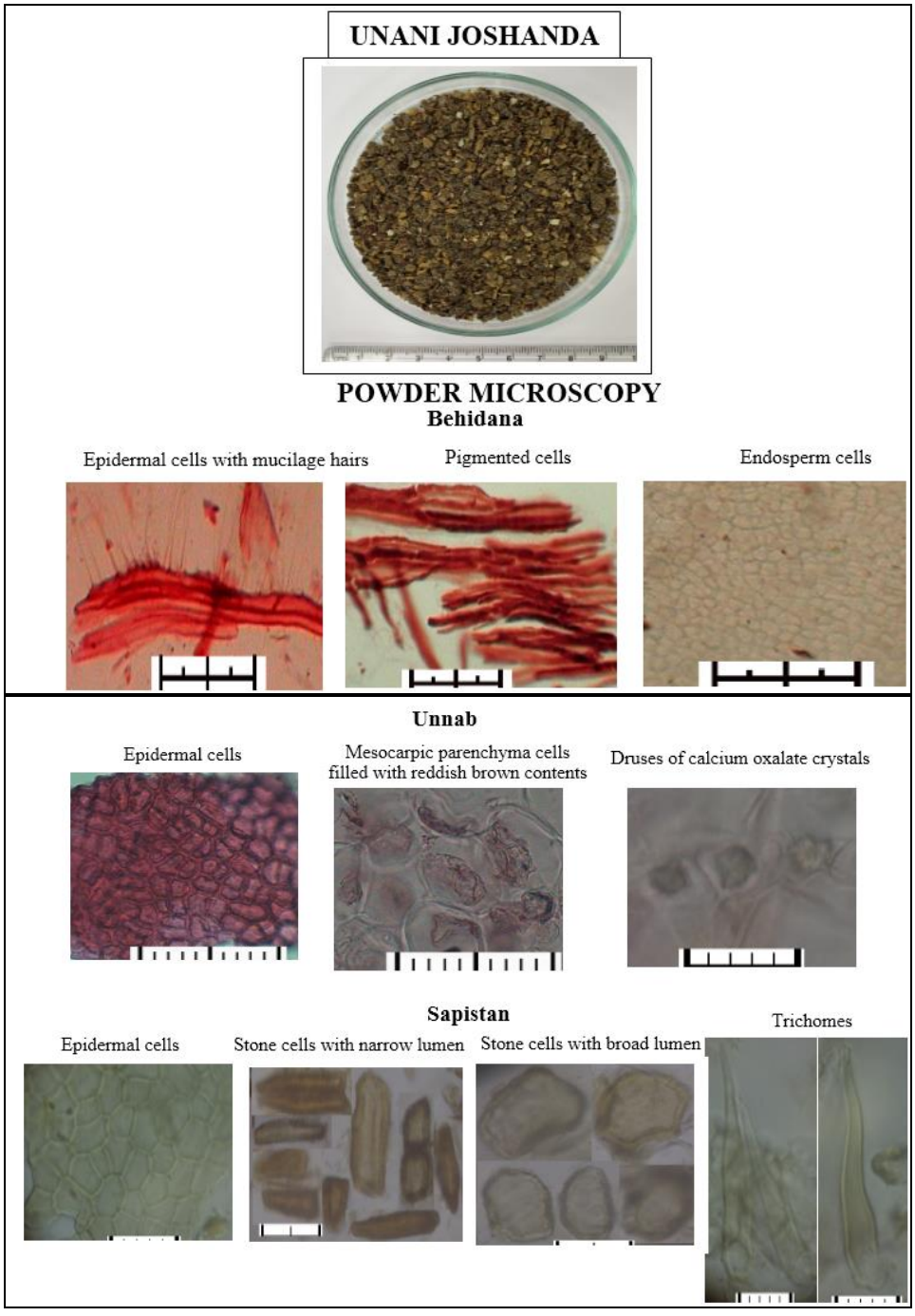


Fig 1: Powder Microscopy of Unani Joshanda (Behidana, Unnab and Sapistan)

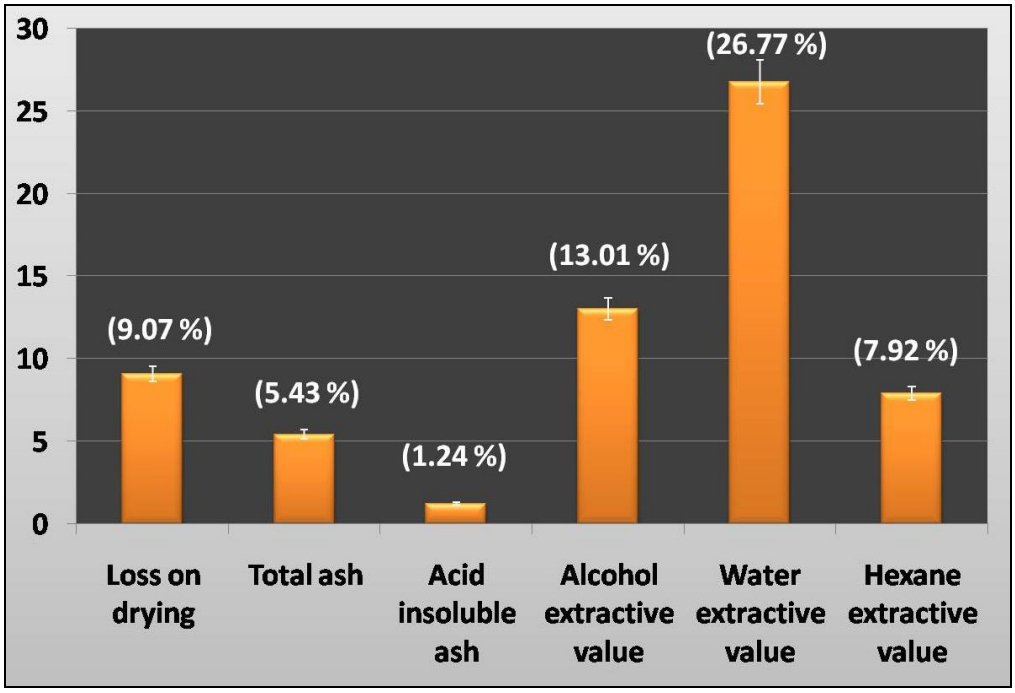
3.2 Physicochemical Analysis

Physicochemical analysis has been done to evaluate the purity and quality of the synthesized drug. In physicochemical evaluation ash values such as total ash value and acid insoluble value, extractive values namely water extractive values, alcohol extractive values and hexane extractive values, volatile oil content, loss on drying and pH were determined are shown in Table 5, Graph 1. Ash values are used to determine the presence or absence of inorganic matter of the drug. Extractive values index the purity of the drug and also helps to determine the adulteration and variations in the chemical constituents because it leads to the change in extractive values. Loss on drying was performed to calculate the amount of water and volatile matter in the drug, which is removed during drying process.

For Unani Joshanda, the organoleptic characteristics i.e appearance, color, smell and taste were found to be brown color, mucilaginous and sweet taste and characteristic its own odour. The total ash value was found to be 5.43% and the acid insoluble ash value was found to be 1.24%. The extractive values such as water extractive value, alcohol extractive value and hexane extractive value were found to be 26.77%, 13.01% and 7.92%. The loss on drying was found to be 9.07% and volatile oil content was found to be 0.02%. The pH of the drug was determined in 5% aqueous solution and the values were found to be 6.0, respectively.

Table 5: Physicochemical parameter of Unani Joshanda

S. No.	Parameters Analyzed	Results
1	Loss on drying (%)	9.07%
2	Ash values (%)	
	Total ash	5.43%
	Acid insoluble ash	1.24%
3	Extractives (%) (cold percolation method)	
	Alcohol soluble matter	13.01%
	Water soluble matter	26.77%
	Hexane soluble matter	7.92%
4	pH of 10% aqueous solution	6.0
5	Volatile Oil Content (%)	0.02%



Graph 1: Physicochemical parameters

3.3 Thin Layer Chromatography of Unani Joshanda

Thin layer chromatography is one of the important parameter used to analyze the quality and purity of the drug and also used to detect the adulteration in the drugs. Moreover, the HPTLC method used to separate the presence of chemical constituents in the drug. It is one of the most efficient and simplest analytical method for qualitative and quantitative analysis of the drug. In this study, the prepared Unani Joshanda drug was carried out in two different solvent such as alcohol and chloroform extract. In thin layer chromatography major spots were shown in chloroform extract when compare to alcohol extract at 254 nm, 366 nm and VSare shown in Figure 2, 8, 14, 15, 20 & 26.HPTLC finger print regions and densitometric chromatogram of the samples of the drug are scanned at 254 and 366 nm. In both chloroform and alcohol extracts the HPTLC finger print profile shows a sharp and symmetrical peaks were obtained and shown in Figure 3-12 & 16-24. Densitometry is an instrumental technique that is more accurate than visual. In this method the resolved spots are scanned and their densities were determined with a densitometer. Figure 7, 13, 19 & 25 shows the densitometric chromatogram of both chloroform and alcohol extracts at two different wavelength of 254 and 366 nm.

The study reveals the TLC of Unani Joshanda (alcohol extract), major spots at 254 nm, 366 nm and VS are shown below.

3.3.1 Thin Layer Chromatography Alcohol Extract

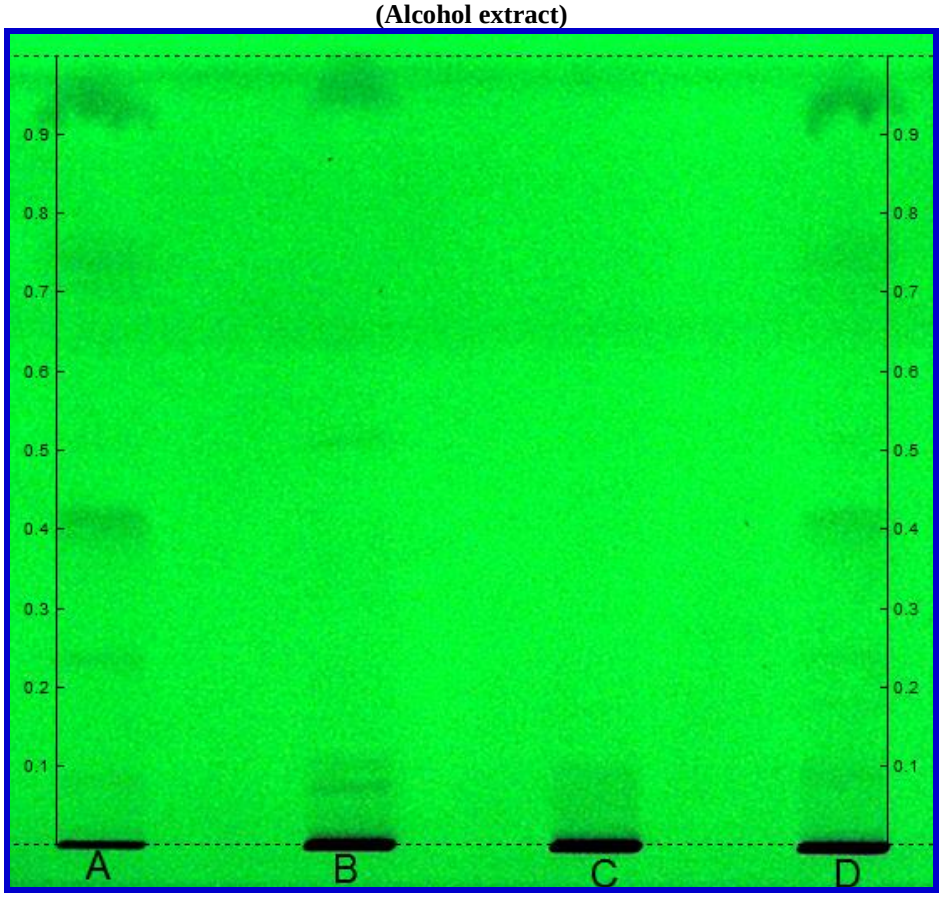
3.3.1.1 Unani Joshanda: After development allows the plate to dry in air and examine under UV (254 nm), it shows major spots at R_f0.96, 0.74, 0.42, 0.24, 0.18 and 0.08 (Green). Under UV (366 nm), it shows major spots at R_f0.94 (Light blue), 0.51 (Light red), 0.46 (Grey) and 0.09 (Blue). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots at R_f 0.93 (Dark violet), 0.80, 0.75, 0.67, 0.55, 0.51, 0.42 (Grey), 0.38 (Dark violet), 0.18 and 0.09 (Grey).

3.3.1.2 Behidana: After development allows the plate to dry in air and examine under UV (254 nm), it shows major spots at R_f0.96 (Green), 0.73 (Light green) 0.42, 0.24 (Green) and 0.08 (Light green). Under UV (366 nm), it shows major spots at R_f0.93 (Blue) and 0.46 (Grey). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots at R_f 0.94 (Dark violet), 0.78, 0.73, 0.55, 0.51, 0.42 (Grey) and 0.36 (Violet).

3.3.1.3 Sapistan: After development allows the plate to dry in air and examine under UV (254 nm), it shows major spots at R_f0.97, 0.52, 0.10 and 0.08 (Green). Under UV (366 nm), it shows major spots at R_f0.95 (Blue) and 0.46 (Fluorescent blue). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots at R_f0.94 (Dark violet), 0.78, 0.75, 0.49, 0.43, (Grey), 0.36 (Violet), 0.11 and 0.08(Grey).

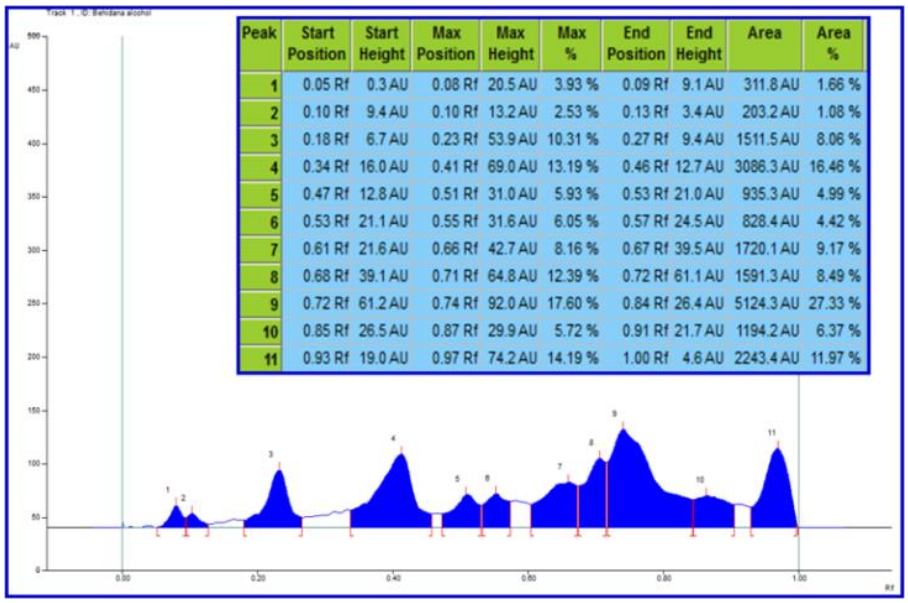
3.3.1.4 Unnab: After development allows the plate to dry in air and examine under UV (254 nm), it shows major spots at R_f0.66 (Light green), 0.09 and 0.06 (Green). Under UV (366 nm), it shows major spots at R_f0.95 (Blue), 0.46 (Light blue) and 0.09 (Light grey). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots at R_f0.96, 0.49, 0.43 (Grey), 0.36 (Violet), 0.31 (Grey), 0.18 (Dark violet), 0.16 and 0.09 (Grey).

Thin Layer Chromatography
(Alcohol extract)



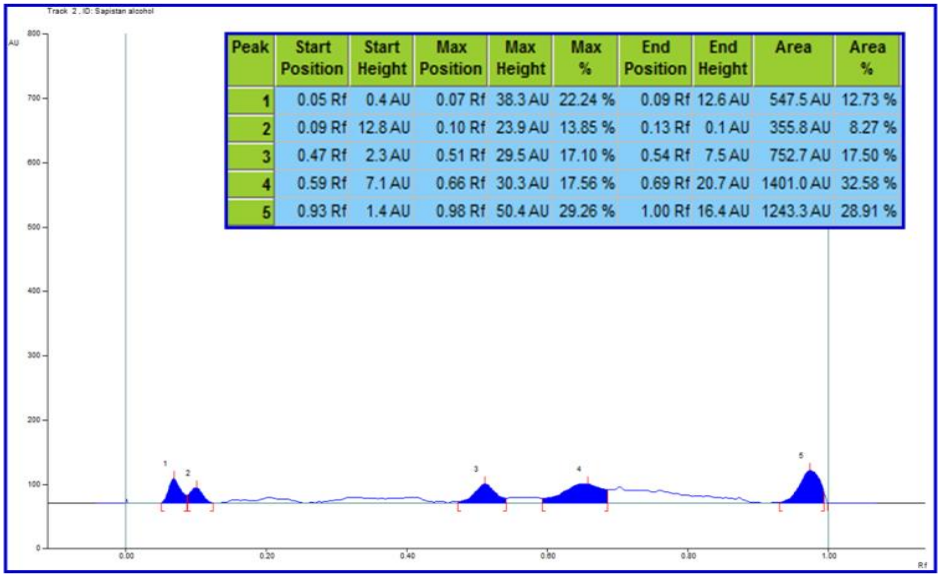
UV 254 nm

Fig 2: Solvent System: Toluene: Ethyl acetate: Formic acid (8.2: 1.8: 0.4) 15 µl Track A: Behidana; Track B: Sapistan; Track C: Unnab; Track D: Unani Joshanda



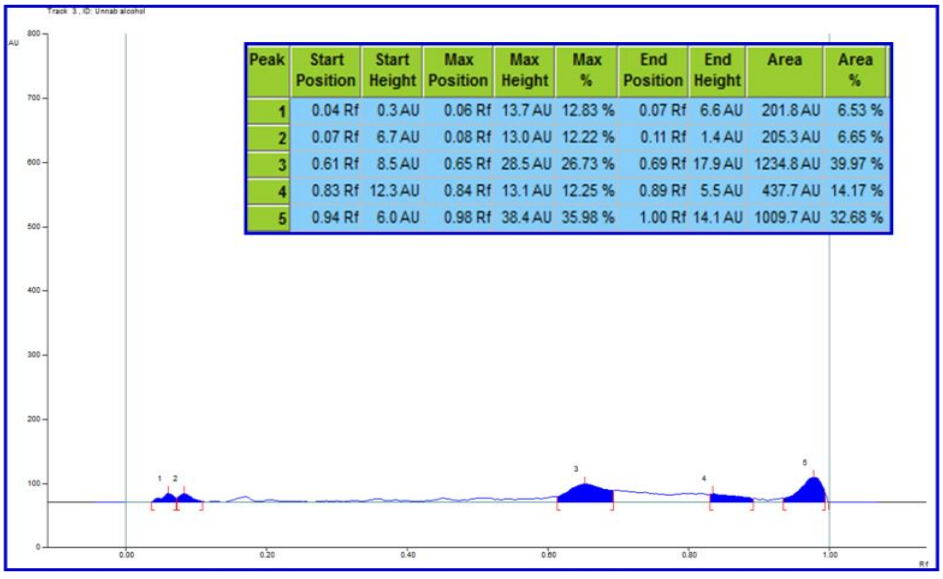
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.3 AU	0.08 Rf	20.5 AU	3.93 %	0.09 Rf	9.1 AU	311.8 AU	1.66 %
2	0.10 Rf	9.4 AU	0.10 Rf	13.2 AU	2.53 %	0.13 Rf	3.4 AU	203.2 AU	1.08 %
3	0.18 Rf	6.7 AU	0.23 Rf	53.9 AU	10.31 %	0.27 Rf	9.4 AU	1511.5 AU	8.06 %
4	0.34 Rf	16.0 AU	0.41 Rf	69.0 AU	13.19 %	0.46 Rf	12.7 AU	3086.3 AU	16.46 %
5	0.47 Rf	12.8 AU	0.51 Rf	31.0 AU	5.93 %	0.53 Rf	21.0 AU	935.3 AU	4.99 %
6	0.53 Rf	21.1 AU	0.55 Rf	31.6 AU	6.05 %	0.57 Rf	24.5 AU	828.4 AU	4.42 %
7	0.61 Rf	21.6 AU	0.66 Rf	42.7 AU	8.16 %	0.67 Rf	39.5 AU	1720.1 AU	9.17 %
8	0.68 Rf	39.1 AU	0.71 Rf	64.8 AU	12.39 %	0.72 Rf	61.1 AU	1591.3 AU	8.49 %
9	0.72 Rf	61.2 AU	0.74 Rf	92.0 AU	17.60 %	0.84 Rf	26.4 AU	5124.3 AU	27.33 %
10	0.85 Rf	26.5 AU	0.87 Rf	29.9 AU	5.72 %	0.91 Rf	21.7 AU	1194.2 AU	6.37 %
11	0.93 Rf	19.0 AU	0.97 Rf	74.2 AU	14.19 %	1.00 Rf	4.6 AU	2243.4 AU	11.97 %

Fig 3: HPTLC finger print and R_f values of Behidana in Alcohol extract at 254 nm (Absorbance mode)



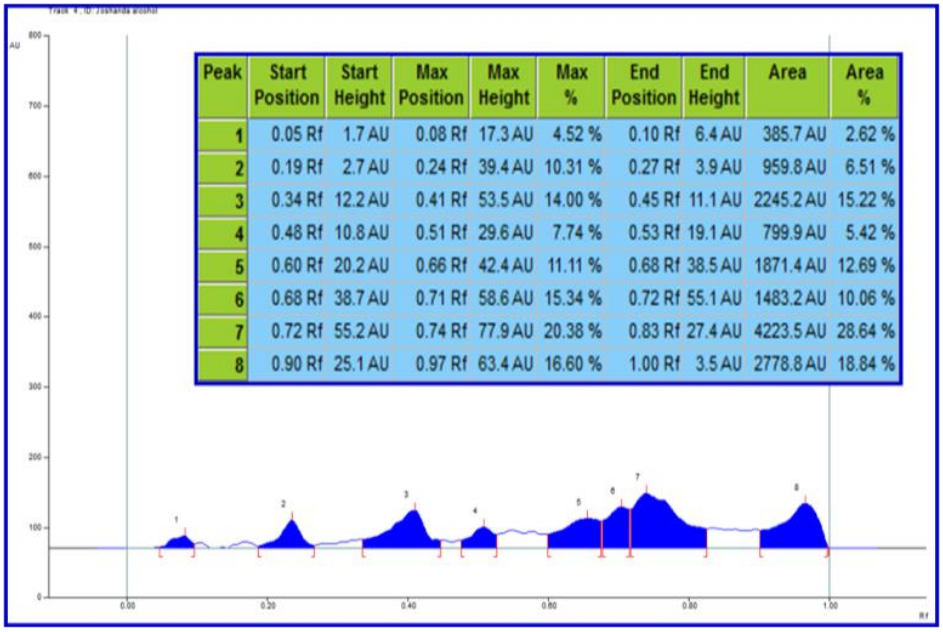
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.4 AU	0.07 Rf	38.3 AU	22.24 %	0.09 Rf	12.6 AU	547.5 AU	12.73 %
2	0.09 Rf	12.8 AU	0.10 Rf	23.9 AU	13.85 %	0.13 Rf	0.1 AU	355.8 AU	8.27 %
3	0.47 Rf	2.3 AU	0.51 Rf	29.5 AU	17.10 %	0.54 Rf	7.5 AU	752.7 AU	17.50 %
4	0.59 Rf	7.1 AU	0.66 Rf	30.3 AU	17.56 %	0.69 Rf	20.7 AU	1401.0 AU	32.58 %
5	0.93 Rf	1.4 AU	0.98 Rf	50.4 AU	29.26 %	1.00 Rf	16.4 AU	1243.3 AU	28.91 %

Fig 4: HPTLC finger print and R_f values of Sapistan in Alcohol extract at 254 nm (Absorbance mode)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.3 AU	0.06 Rf	13.7 AU	12.83 %	0.07 Rf	6.6 AU	201.8 AU	6.53 %
2	0.07 Rf	6.7 AU	0.08 Rf	13.0 AU	12.22 %	0.11 Rf	1.4 AU	205.3 AU	6.65 %
3	0.61 Rf	8.5 AU	0.65 Rf	28.5 AU	26.73 %	0.69 Rf	17.9 AU	1234.8 AU	39.97 %
4	0.83 Rf	12.3 AU	0.84 Rf	13.1 AU	12.25 %	0.89 Rf	5.5 AU	437.7 AU	14.17 %
5	0.94 Rf	6.0 AU	0.98 Rf	38.4 AU	35.98 %	1.00 Rf	14.1 AU	1009.7 AU	32.68 %

Fig 5: HPTLC finger print and R_f values of Unnab in Alcohol extract at 254 nm (Absorbance mode)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	1.7 AU	0.08 Rf	17.3 AU	4.52 %	0.10 Rf	6.4 AU	385.7 AU	2.62 %
2	0.19 Rf	2.7 AU	0.24 Rf	39.4 AU	10.31 %	0.27 Rf	3.9 AU	959.8 AU	6.51 %
3	0.34 Rf	12.2 AU	0.41 Rf	53.5 AU	14.00 %	0.45 Rf	11.1 AU	2245.2 AU	15.22 %
4	0.48 Rf	10.8 AU	0.51 Rf	29.6 AU	7.74 %	0.53 Rf	19.1 AU	799.9 AU	5.42 %
5	0.60 Rf	20.2 AU	0.66 Rf	42.4 AU	11.11 %	0.68 Rf	38.5 AU	1871.4 AU	12.69 %
6	0.68 Rf	38.7 AU	0.71 Rf	58.6 AU	15.34 %	0.72 Rf	55.1 AU	1483.2 AU	10.06 %
7	0.72 Rf	55.2 AU	0.74 Rf	77.9 AU	20.38 %	0.83 Rf	27.4 AU	4223.5 AU	28.64 %
8	0.90 Rf	25.1 AU	0.97 Rf	63.4 AU	16.60 %	1.00 Rf	3.5 AU	2778.8 AU	18.84 %

Fig 6: HPTLC finger print and R_f values of Unani Joshanda in Alcohol extract at 254 nm (Absorbance mode)

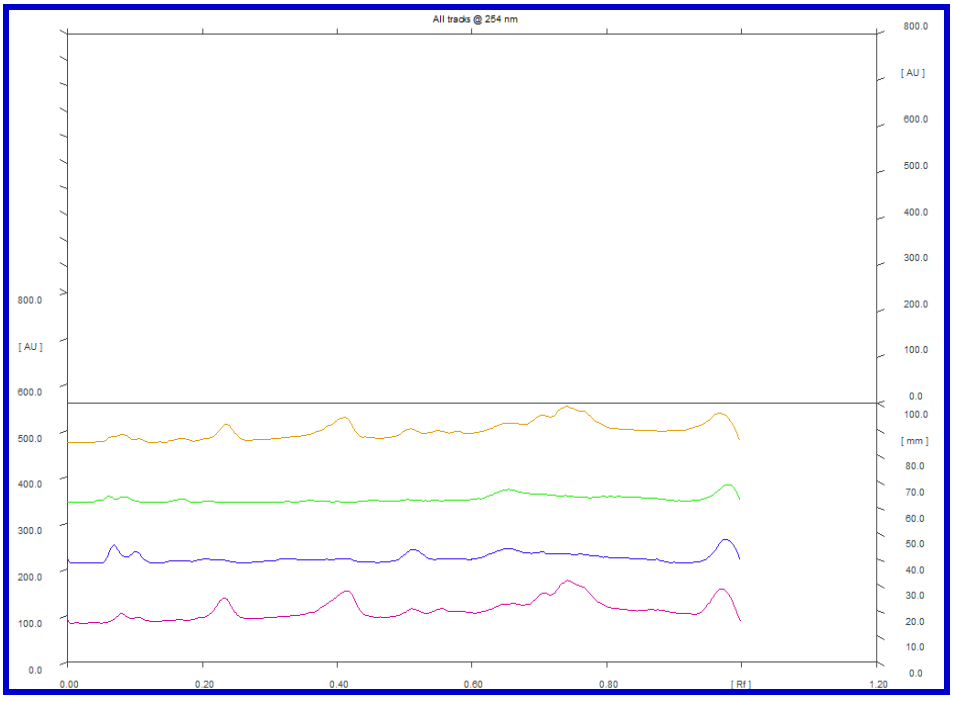
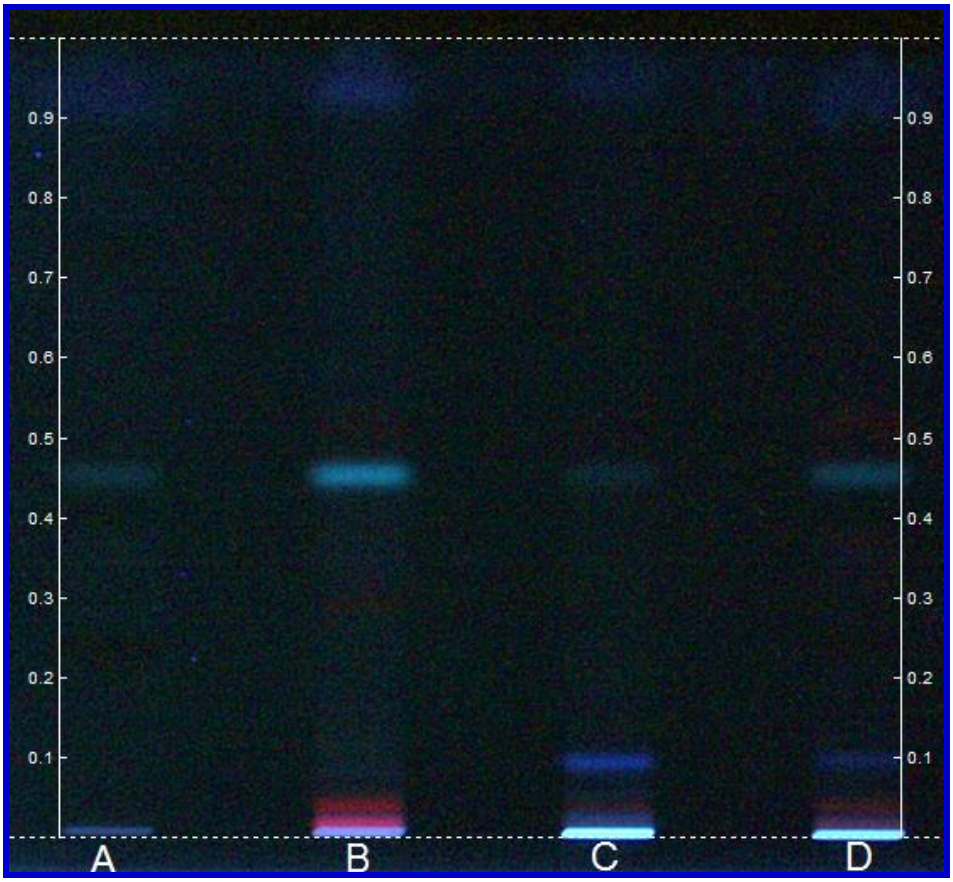
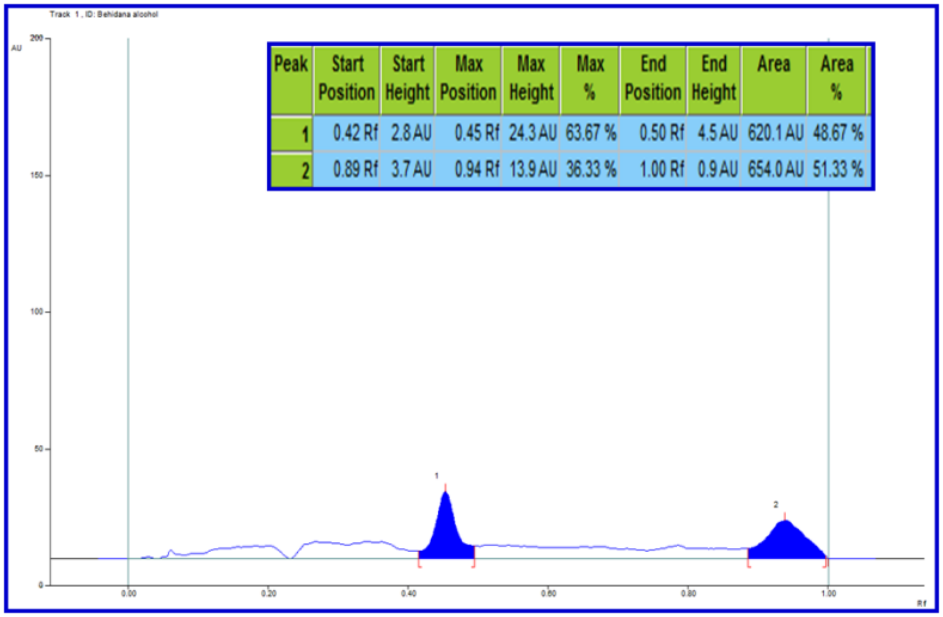


Fig 7: Densitometric chromatogram of Behidana, Sapistan, Unnab& Unani Joshanda in Alcohol extract at 254 nm (Absorbance mode)



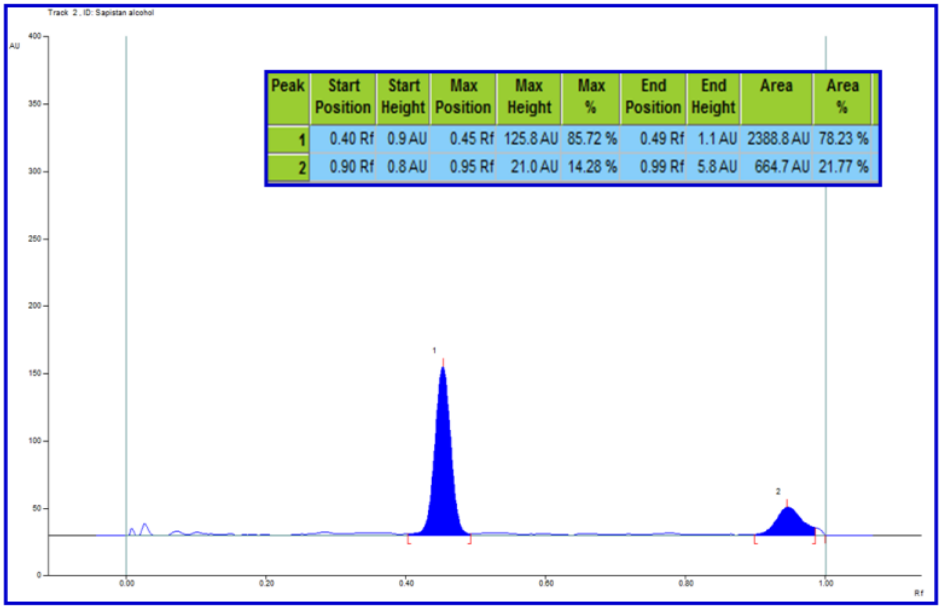
UV 366 nm

Fig 8: Solvent System: Toluene : Ethyl acetate : Formic acid (8.2: 1.8: 0.4) 15 µl Track A: Behidana; Track B: Sapistan; Track C: Unnab; Track D: Unani Joshanda



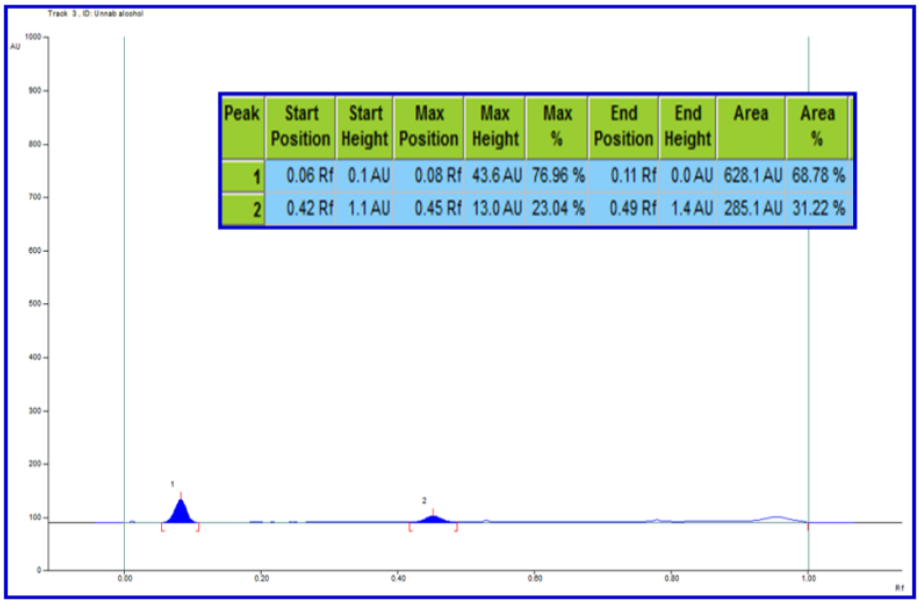
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.42 Rf	2.8 AU	0.45 Rf	24.3 AU	63.67 %	0.50 Rf	4.5 AU	620.1 AU	48.67 %
2	0.89 Rf	3.7 AU	0.94 Rf	13.9 AU	36.33 %	1.00 Rf	0.9 AU	654.0 AU	51.33 %

Fig 9: HPTLC finger print and R_f values of Behidana in Alcohol extract at 366 nm (Fluorescence mode)



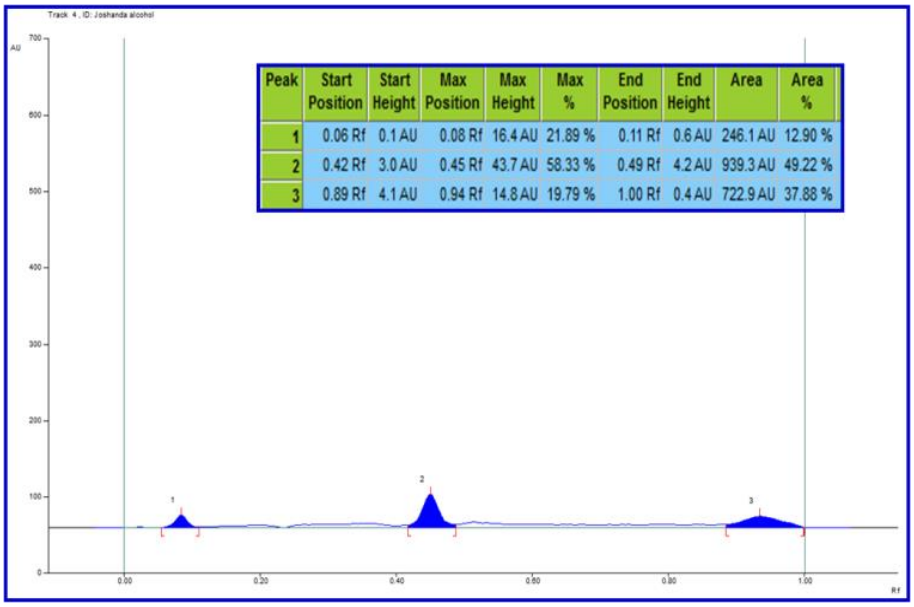
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.40 Rf	0.9 AU	0.45 Rf	125.8 AU	85.72 %	0.49 Rf	1.1 AU	2388.8 AU	78.23 %
2	0.90 Rf	0.8 AU	0.95 Rf	21.0 AU	14.28 %	0.99 Rf	5.8 AU	664.7 AU	21.77 %

Fig 10: HPTLC finger print and R_f values of Sapistan in Alcohol extract at 366 nm (Fluorescence mode)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	0.1 AU	0.08 Rf	43.6 AU	76.96 %	0.11 Rf	0.0 AU	628.1 AU	68.78 %
2	0.42 Rf	1.1 AU	0.45 Rf	13.0 AU	23.04 %	0.49 Rf	1.4 AU	285.1 AU	31.22 %

Fig 11: HPTLC finger print and R_f values of Unnab in Alcohol extract at 366 nm (Fluorescence mode)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	0.1 AU	0.08 Rf	16.4 AU	21.89 %	0.11 Rf	0.6 AU	246.1 AU	12.90 %
2	0.42 Rf	3.0 AU	0.45 Rf	43.7 AU	58.33 %	0.49 Rf	4.2 AU	939.3 AU	49.22 %
3	0.89 Rf	4.1 AU	0.94 Rf	14.8 AU	19.79 %	1.00 Rf	0.4 AU	722.9 AU	37.88 %

Fig 12: HPTLC finger print and R_f values of Unani Joshanda in Alcohol extract at 366nm (Fluorescence mode)

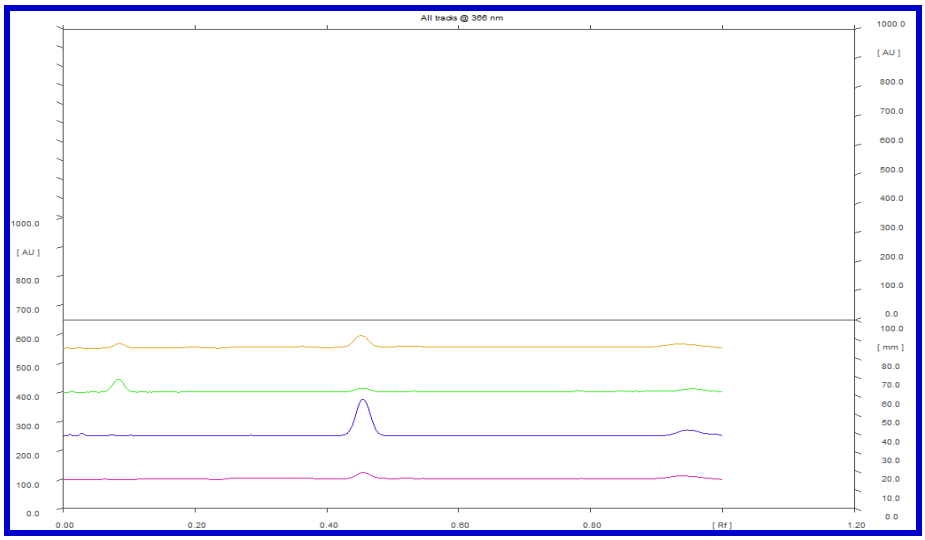
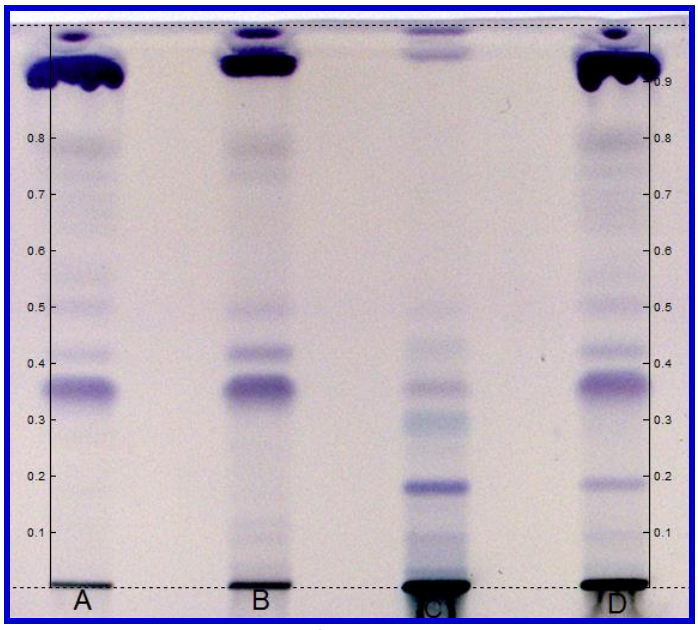


Fig 13: Densitometric chromatogram of Behidana, Sapistan, Unnab& Unani Joshanda in Alcohol extract at 366 nm (Fluorescence mode)



V-S Reagent

Fig 14: Solvent System: Toluene : Ethyl acetate : Formic acid (8.2: 1.8: 0.4) 15 µl Track A: Behidana; Track B: Sapistan; Track C: Unnab; Track D: Unani Joshanda

3.3.2 Thin Layer Chromatography Chloroform Extract
The study reveals the TLC of Unani Joshanda (chloroform extract), major spots at 254 nm, 366 nm and VS are shown below.

3.3.2.1 Unani Joshanda: After development allows the plate to dry in air and examine under UV (254 nm), it shows major spots at R_f0.91, 0.58 (Dark green), 0.41 and 0.23 (Green). Under UV (366 nm), it shows major spots at R_f0.88, (Blue), 0.68 (Light violet), 0.59 (Fluorescent blue), 0.46 (Light red), 0.29 (Light yellow), 0.18 (Blue), 0.16 and 0.07 (Red). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots at R_f 0.97 (Dark grey), 0.59 (Grey), 0.52 (Dark grey), 0.47 (Violet), 0.35, 0.21 and 0.12 (Grey).

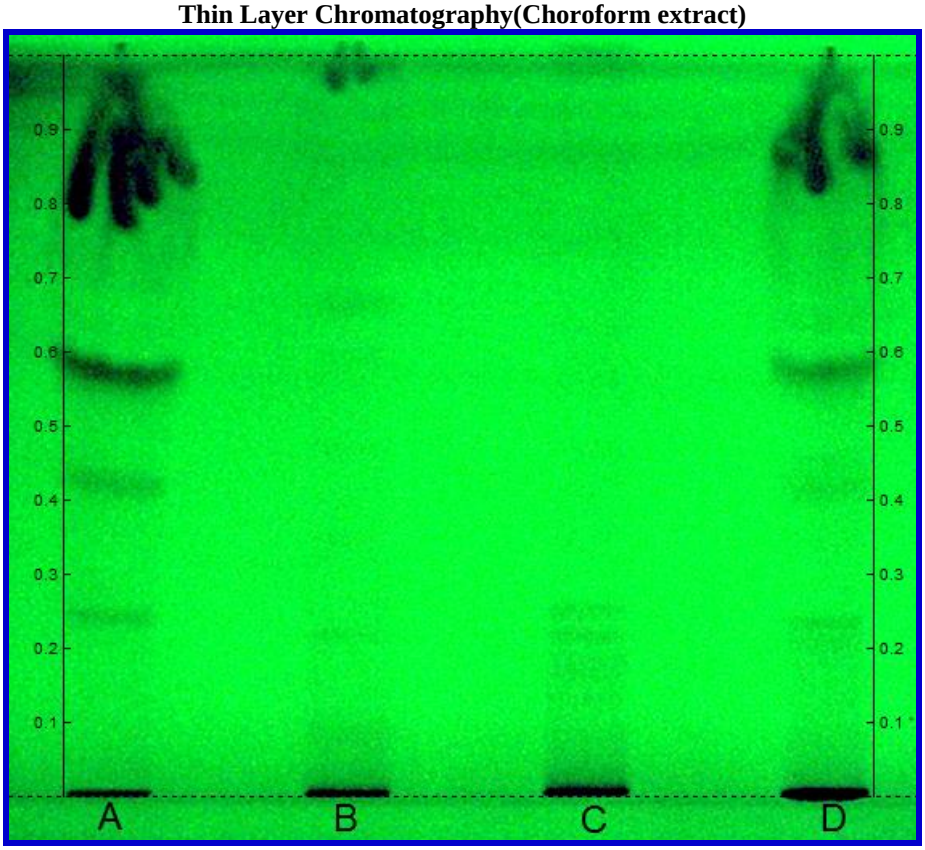
3.3.2.2 Behidana: After development allows the plate to dry in air and examine under UV (254 nm), it shows major spots at R_f0.96, 0.57 (Dark green), 0.42 and 0.26 (Green). Under UV (366 nm), it shows major spots at R_f0.96 (Blue), 0.68 (Light violet), 0.59 (Fluorescent blue), 0.42 (Dark brown), and 0.20 (Light grey). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots at R_f 0.97 (Dark grey), 0.59 (Grey), 0.54 (Dark grey), 0.47 (Violet), 0.24, 0.14 and 0.09 (Light grey).

3.3.2.3 Sapistan: After development allows the plate to dry in air and examine under UV (254 nm), it shows major spots at R_f0.96 (Dark green), 0.66, 0.45, 0.34, 0.21 and 0.08 (Green). Under UV (366 nm), it shows major spots at R_f0.97, 0.90 (Blue), 0.70 (Violet), 0.61 (Fluorescent blue), 0.48, 0.35, 0.15 and 0.07 (Red). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots at R_f0.85, 0.60, 0.52 (Grey), 0.48 (Violet), 0.25 and 0.12 (Grey).

3.3.2.4 Unnab: After development allows the plate to dry in air and examine under UV (254 nm), it shows major spots at R_f0.97, 0.27, 0.25, 0.20 and 0.17 (Green). Under UV (366 nm), it shows major spots at R_f0.96, 0.90 (Blue), 0.70 (Light violet), 0.61 (Blue), 0.31 (Light yellow), 0.17 (Fluorescent

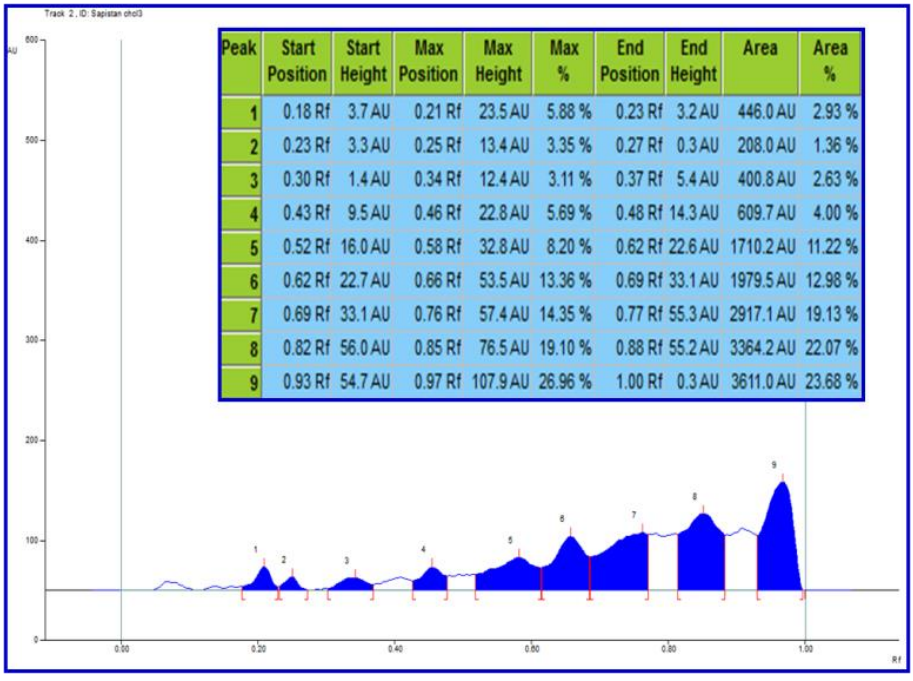
blue), 0.14 (Red) and 0.06 (Blue). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots at R_f0.85, 0.62, 0.52 (Light grey), 0.36 (Dark grey), 0.24, 0.16 and 0.13 (Grey).

Thin Layer Chromatography(Choroform extract)



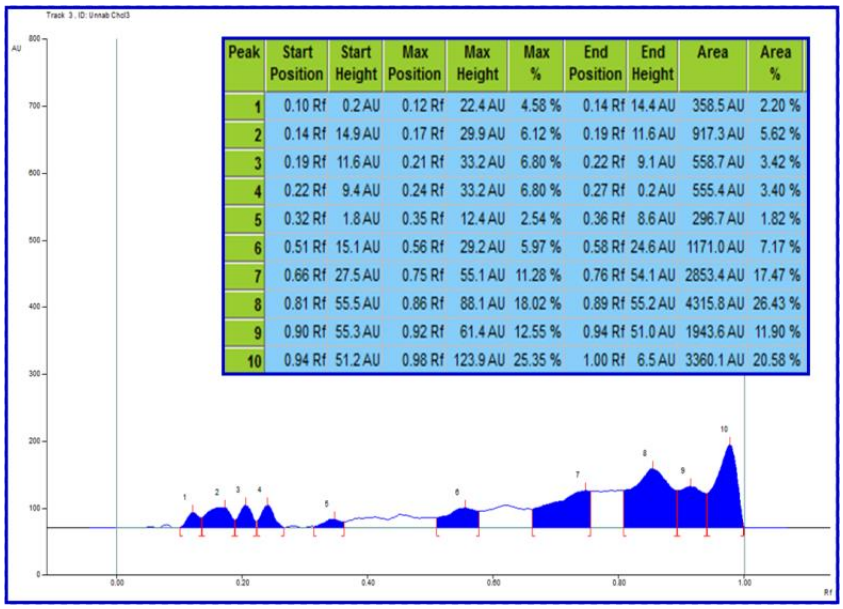
UV 254 nm

Fig 15: Solvent System: Toluene : Ethyl acetate : Formic acid (8.2: 1.8: 0.4) 10 µl Track A: Behidana; Track B: Sapistan; Track C: Unnab; Track D: Unani Joshanda



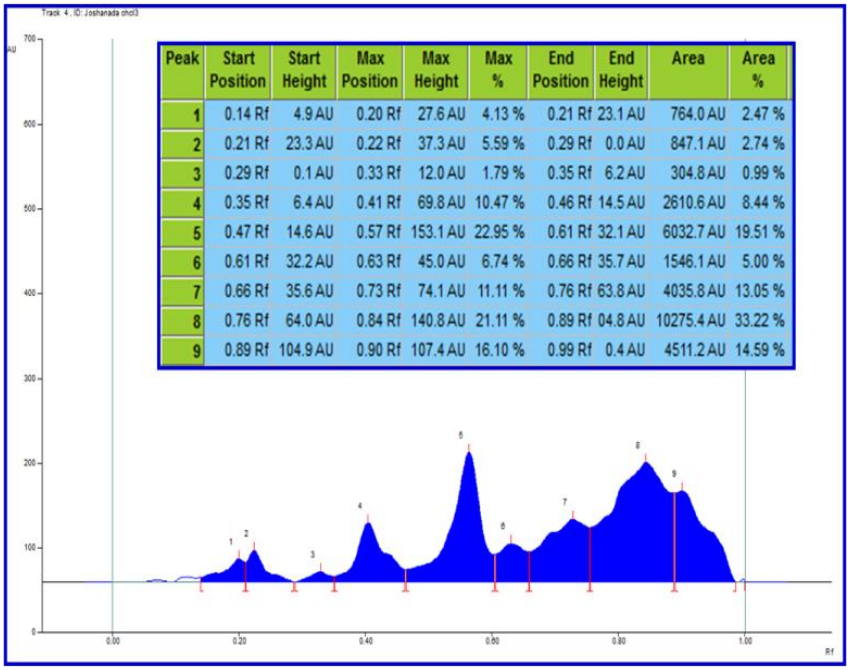
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.18 Rf	3.7 AU	0.21 Rf	23.5 AU	5.88 %	0.23 Rf	3.2 AU	446.0 AU	2.93 %
2	0.23 Rf	3.3 AU	0.25 Rf	13.4 AU	3.35 %	0.27 Rf	0.3 AU	208.0 AU	1.36 %
3	0.30 Rf	1.4 AU	0.34 Rf	12.4 AU	3.11 %	0.37 Rf	5.4 AU	400.8 AU	2.63 %
4	0.43 Rf	9.5 AU	0.46 Rf	22.8 AU	5.69 %	0.48 Rf	14.3 AU	609.7 AU	4.00 %
5	0.52 Rf	16.0 AU	0.58 Rf	32.8 AU	8.20 %	0.62 Rf	22.6 AU	1710.2 AU	11.22 %
6	0.62 Rf	22.7 AU	0.66 Rf	53.5 AU	13.36 %	0.69 Rf	33.1 AU	1979.5 AU	12.98 %
7	0.69 Rf	33.1 AU	0.76 Rf	57.4 AU	14.35 %	0.77 Rf	55.3 AU	2917.1 AU	19.13 %
8	0.82 Rf	56.0 AU	0.85 Rf	76.5 AU	19.10 %	0.88 Rf	55.2 AU	3364.2 AU	22.07 %
9	0.93 Rf	54.7 AU	0.97 Rf	107.9 AU	26.96 %	1.00 Rf	0.3 AU	3611.0 AU	23.68 %

Fig 16: HPTLC finger print of Sapistan in Chloroform extract at 254 nm (Absorbance mode)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.10 Rf	0.2 AU	0.12 Rf	22.4 AU	4.58 %	0.14 Rf	14.4 AU	358.5 AU	2.20 %
2	0.14 Rf	14.9 AU	0.17 Rf	29.9 AU	6.12 %	0.19 Rf	11.6 AU	917.3 AU	5.62 %
3	0.19 Rf	11.6 AU	0.21 Rf	33.2 AU	6.80 %	0.22 Rf	9.1 AU	558.7 AU	3.42 %
4	0.22 Rf	9.4 AU	0.24 Rf	33.2 AU	6.80 %	0.27 Rf	0.2 AU	555.4 AU	3.40 %
5	0.32 Rf	1.8 AU	0.35 Rf	12.4 AU	2.54 %	0.36 Rf	8.6 AU	296.7 AU	1.82 %
6	0.51 Rf	15.1 AU	0.56 Rf	29.2 AU	5.97 %	0.58 Rf	24.6 AU	1171.0 AU	7.17 %
7	0.66 Rf	27.5 AU	0.75 Rf	55.1 AU	11.28 %	0.76 Rf	54.1 AU	2853.4 AU	17.47 %
8	0.81 Rf	55.5 AU	0.86 Rf	88.1 AU	18.02 %	0.89 Rf	55.2 AU	4315.8 AU	26.43 %
9	0.90 Rf	55.3 AU	0.92 Rf	61.4 AU	12.55 %	0.94 Rf	51.0 AU	1943.6 AU	11.90 %
10	0.94 Rf	51.2 AU	0.98 Rf	123.9 AU	25.35 %	1.00 Rf	6.5 AU	3360.1 AU	20.58 %

Fig 17: HPTLC finger print of Unnab in Chloroform extract at 254 nm (Absorbance mode)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.14 Rf	4.9 AU	0.20 Rf	27.6 AU	4.13 %	0.21 Rf	23.1 AU	764.0 AU	2.47 %
2	0.21 Rf	23.3 AU	0.22 Rf	37.3 AU	5.59 %	0.29 Rf	0.0 AU	847.1 AU	2.74 %
3	0.29 Rf	0.1 AU	0.33 Rf	12.0 AU	1.79 %	0.35 Rf	6.2 AU	304.8 AU	0.99 %
4	0.35 Rf	6.4 AU	0.41 Rf	69.8 AU	10.47 %	0.46 Rf	14.5 AU	2610.6 AU	8.44 %
5	0.47 Rf	14.6 AU	0.57 Rf	153.1 AU	22.95 %	0.61 Rf	32.1 AU	6032.7 AU	19.51 %
6	0.61 Rf	32.2 AU	0.63 Rf	45.0 AU	6.74 %	0.66 Rf	35.7 AU	1546.1 AU	5.00 %
7	0.66 Rf	35.6 AU	0.73 Rf	74.1 AU	11.11 %	0.76 Rf	63.8 AU	4035.8 AU	13.05 %
8	0.76 Rf	64.0 AU	0.84 Rf	140.8 AU	21.11 %	0.89 Rf	04.8 AU	10275.4 AU	33.22 %
9	0.89 Rf	104.9 AU	0.90 Rf	107.4 AU	16.10 %	0.99 Rf	0.4 AU	4511.2 AU	14.59 %

Fig 18: HPTLC finger print of Unani Joshanda in Chloroform extract at 254 nm (Absorbance mode)

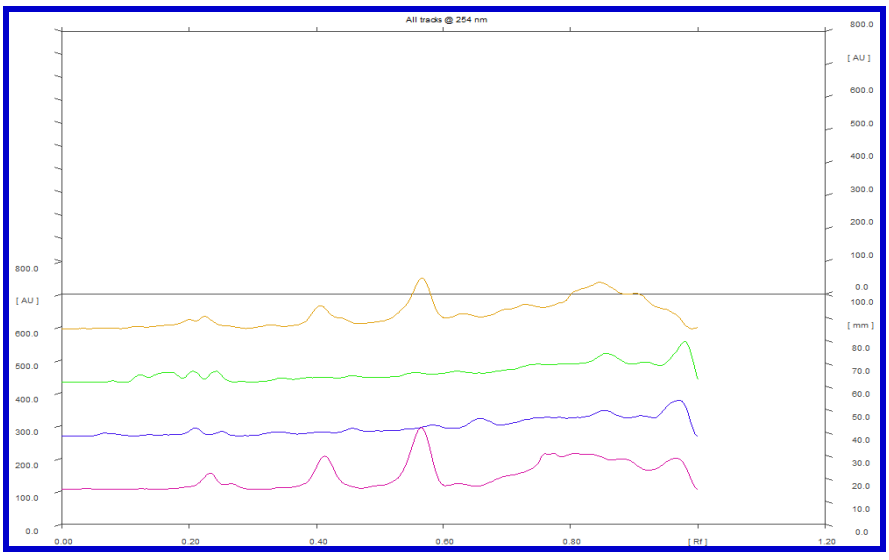
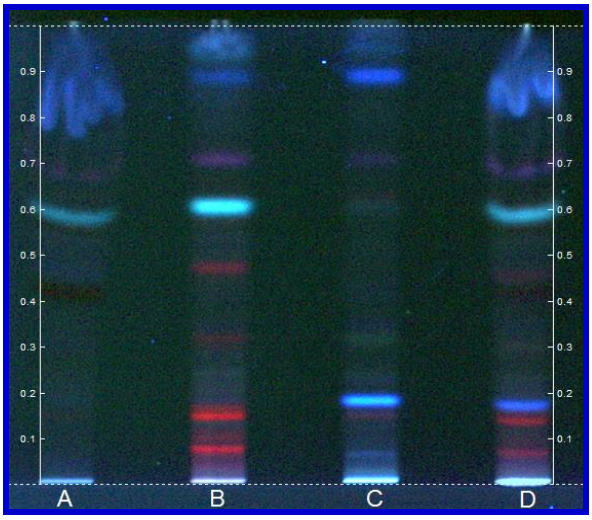
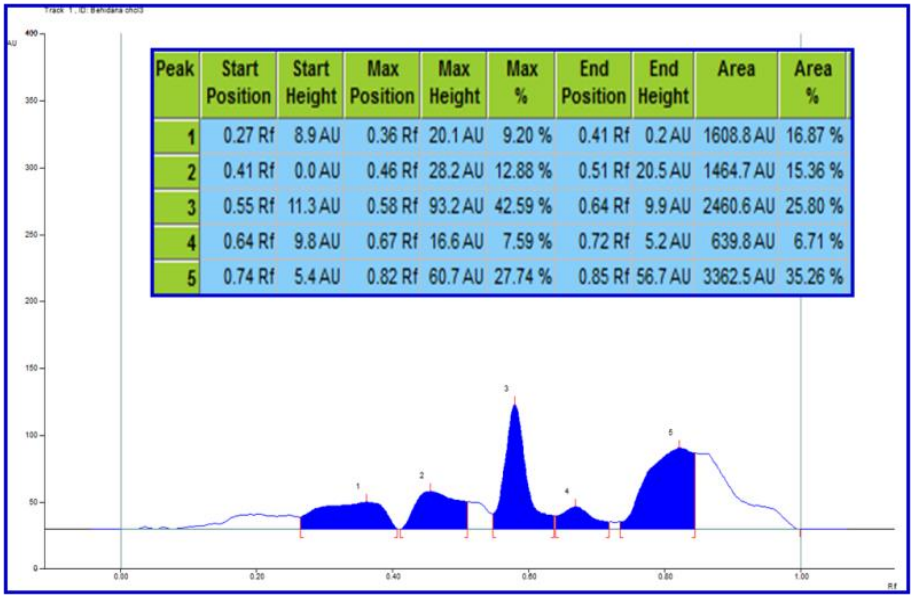


Fig 19: Densitometric chromatogram of Behidana, Sapistan, Unnab& Unani Joshanda in Chloroform extract at 254 nm (Absorbance mode)



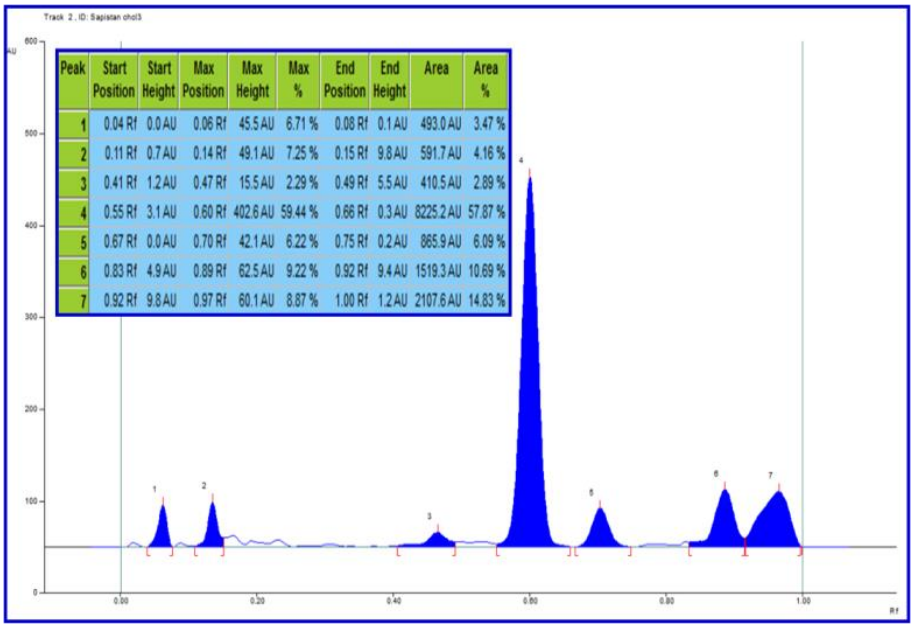
UV 366 nm

Fig 20: Solvent System: Toluene : Ethyl acetate : Formic acid (8.2: 1.8: 0.4) 10 µl Track A: Behidana; Track B: Sapistan; Track C: Unnab; Track D: Unani Joshanda



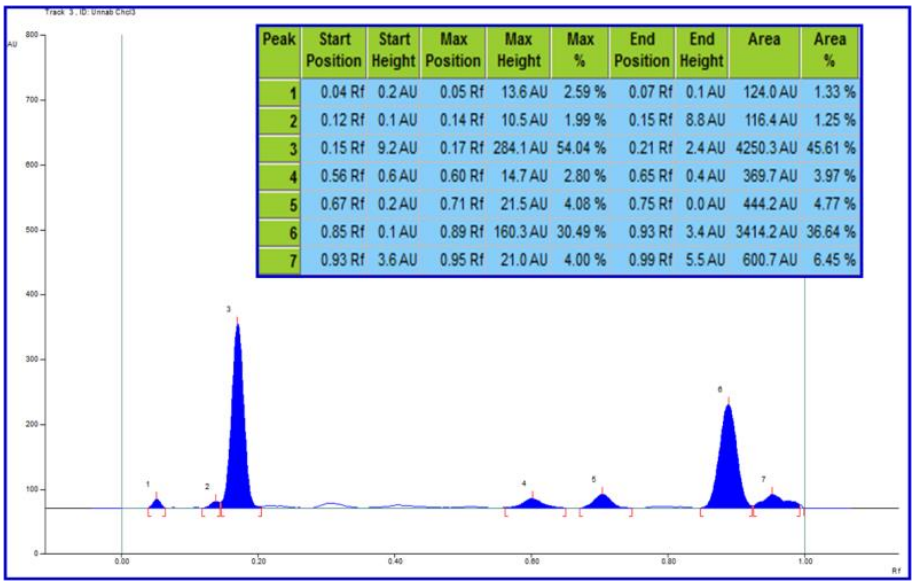
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.27 Rf	8.9 AU	0.36 Rf	20.1 AU	9.20 %	0.41 Rf	0.2 AU	1608.8 AU	16.87 %
2	0.41 Rf	0.0 AU	0.46 Rf	28.2 AU	12.88 %	0.51 Rf	20.5 AU	1464.7 AU	15.36 %
3	0.55 Rf	11.3 AU	0.58 Rf	93.2 AU	42.59 %	0.64 Rf	9.9 AU	2460.6 AU	25.80 %
4	0.64 Rf	9.8 AU	0.67 Rf	16.6 AU	7.59 %	0.72 Rf	5.2 AU	639.8 AU	6.71 %
5	0.74 Rf	5.4 AU	0.82 Rf	60.7 AU	27.74 %	0.85 Rf	56.7 AU	3362.5 AU	35.26 %

Fig 21: HPTLC finger print of Behidana in Chloroform extract at 366 nm (Fluorescence mode)



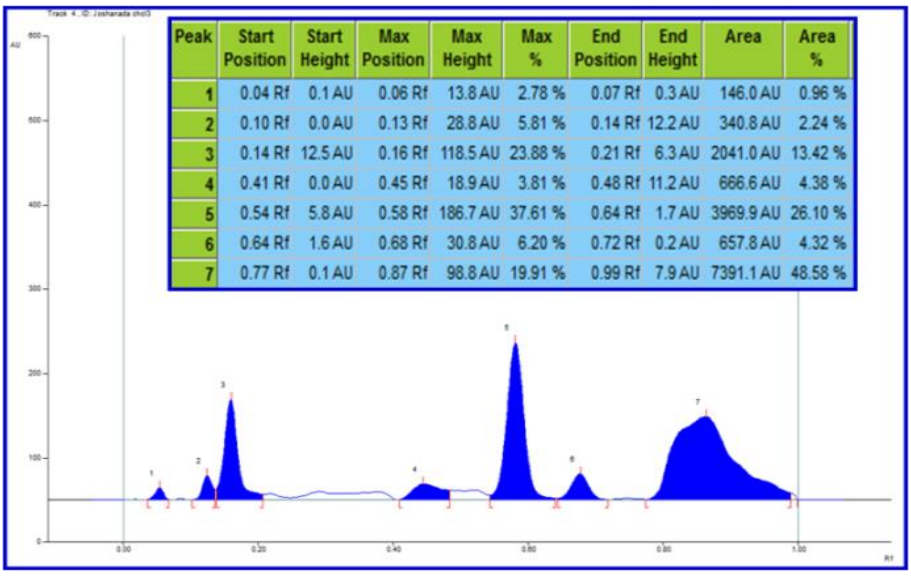
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.0 AU	0.06 Rf	45.5 AU	6.71 %	0.08 Rf	0.1 AU	493.0 AU	3.47 %
2	0.11 Rf	0.7 AU	0.14 Rf	49.1 AU	7.25 %	0.15 Rf	9.8 AU	591.7 AU	4.16 %
3	0.41 Rf	1.2 AU	0.47 Rf	15.5 AU	2.29 %	0.49 Rf	5.5 AU	410.5 AU	2.89 %
4	0.55 Rf	3.1 AU	0.60 Rf	402.6 AU	59.44 %	0.66 Rf	0.3 AU	8225.2 AU	57.87 %
5	0.67 Rf	0.0 AU	0.70 Rf	42.1 AU	6.22 %	0.75 Rf	0.2 AU	865.9 AU	6.09 %
6	0.83 Rf	4.9 AU	0.89 Rf	62.5 AU	9.22 %	0.92 Rf	9.4 AU	1519.3 AU	10.69 %
7	0.92 Rf	9.8 AU	0.97 Rf	60.1 AU	8.87 %	1.00 Rf	1.2 AU	2107.6 AU	14.83 %

Fig 22: HPTLC finger print of Sapistan in Chloroform extract at 366 nm (Fluorescence mode)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.2 AU	0.05 Rf	13.6 AU	2.59 %	0.07 Rf	0.1 AU	124.0 AU	1.33 %
2	0.12 Rf	0.1 AU	0.14 Rf	10.5 AU	1.99 %	0.15 Rf	8.8 AU	116.4 AU	1.25 %
3	0.15 Rf	9.2 AU	0.17 Rf	284.1 AU	54.04 %	0.21 Rf	2.4 AU	4250.3 AU	45.61 %
4	0.56 Rf	0.6 AU	0.60 Rf	14.7 AU	2.80 %	0.65 Rf	0.4 AU	369.7 AU	3.97 %
5	0.67 Rf	0.2 AU	0.71 Rf	21.5 AU	4.08 %	0.75 Rf	0.0 AU	444.2 AU	4.77 %
6	0.85 Rf	0.1 AU	0.89 Rf	160.3 AU	30.49 %	0.93 Rf	3.4 AU	3414.2 AU	36.64 %
7	0.93 Rf	3.6 AU	0.95 Rf	21.0 AU	4.00 %	0.99 Rf	5.5 AU	600.7 AU	6.45 %

Fig 23: HPTLC finger print of Unnab in Chloroform extract at 366 nm (Fluorescence mode)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.1 AU	0.06 Rf	13.8 AU	2.78 %	0.07 Rf	0.3 AU	146.0 AU	0.96 %
2	0.10 Rf	0.0 AU	0.13 Rf	28.8 AU	5.81 %	0.14 Rf	12.2 AU	340.8 AU	2.24 %
3	0.14 Rf	12.5 AU	0.16 Rf	118.5 AU	23.88 %	0.21 Rf	6.3 AU	2041.0 AU	13.42 %
4	0.41 Rf	0.0 AU	0.45 Rf	18.9 AU	3.81 %	0.48 Rf	11.2 AU	666.6 AU	4.38 %
5	0.54 Rf	5.8 AU	0.58 Rf	186.7 AU	37.61 %	0.64 Rf	1.7 AU	3969.9 AU	26.10 %
6	0.64 Rf	1.6 AU	0.68 Rf	30.8 AU	6.20 %	0.72 Rf	0.2 AU	657.8 AU	4.32 %
7	0.77 Rf	0.1 AU	0.87 Rf	98.8 AU	19.91 %	0.99 Rf	7.9 AU	7391.1 AU	48.58 %

Fig 24: HPTLC finger print of Unani Joshanda in Chloroform extract at 366 nm (Fluorescence mode)

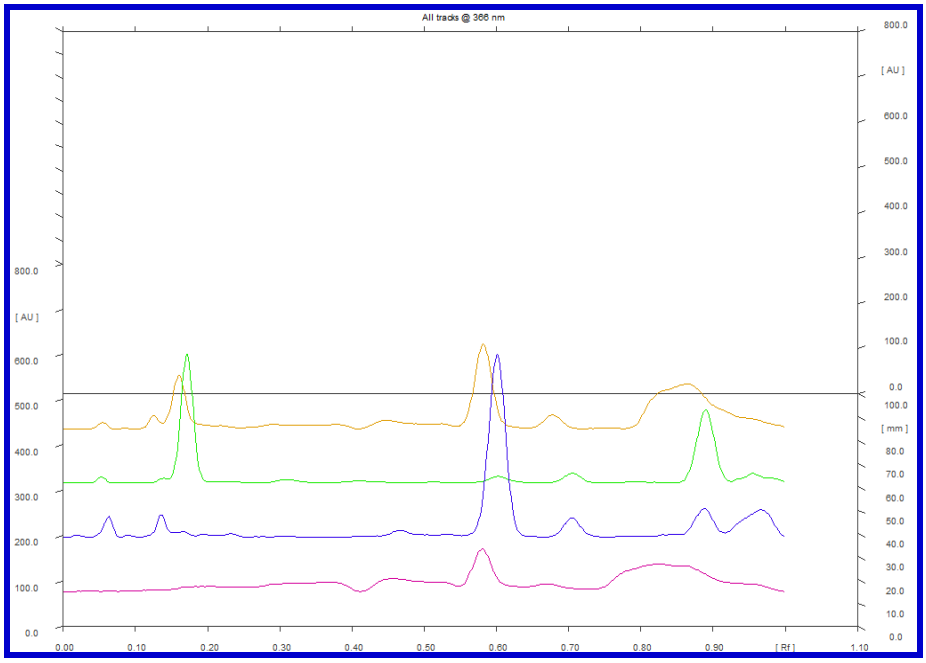
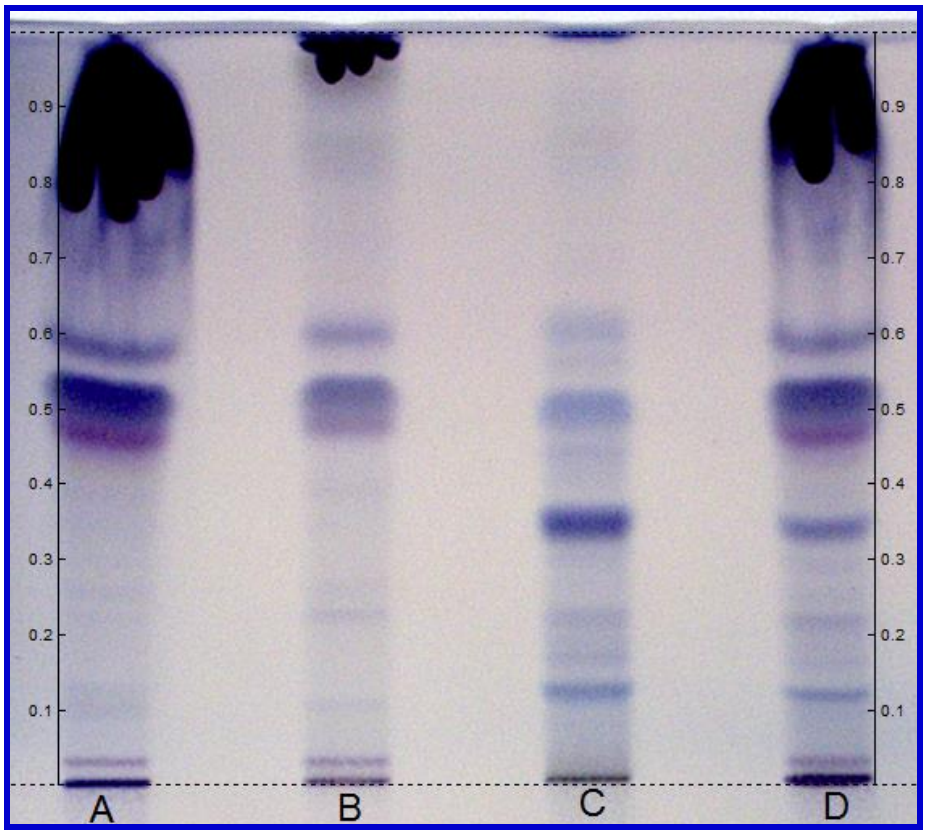


Fig 25: Densitometric chromatogram of Behidana, Sapistan, Unnab& Unani Joshanda in Chloroform extract at 366 nm (Fluorescence mode)



V-S Reagent

Fig 26: Solvent System: Toluene : Ethyl acetate : Formic acid (8.2: 1.8: 0.4) 10 µl Track A: Behidana; Track B: Sapistan; Track C: Unnab; Track D: Unani Joshanda

3.4 Quality Control Parameters

The microbial contents were found to be within the permissible limit. The other parameters such as heavy metals and aflatoxin were not detected from the drug which indicates the drug is free from toxic substances. The results of Microbial load, aflatoxin and heavy metals are tabulated in Table 6.

Table 6: Quality control parametersof Unani Joshanda

S. No.	Name of the Analysis	Results				Permissible Limits as per UPI	Inference
1	**Microbial load**						
	Parameters	**A**	**B**	**C**	**D**		
	Total Microbial Plate count	2×10^2 cfu/g	5×10^3 cfu/g	< 10 cfu/g	7×10^4 cfu/g	10^7cfu/g	Within the permissible limits
	Total Yeast and Mould count	1×10^1 cfu/g	< 10 cfu/g	< 10 cfu/g	2×10^2 cfu/g	10^4cfu/g	
	Specific microorganisms						
	Escherichia coli	Absent	Absent	Absent	Absent	10cfu/gram	
	Salmonella spp.	Absent	Absent	Absent	Absent	None	
	Pseudomonas aeruginosa	Absent	Absent	Absent	Absent	None	
	Staphylococcus aureus	Absent	Absent	Absent	Absent	None	
A: Behidana; B: Sapistan; C: Unnab; D: Unani Joshanda							
2	**Heavy metals**	**Name of the element**		**Results**		**Permissible Limits (ppm) (UPI)**	**Inference**
		Lead		1.11 mg/L		10	Within the permissible limits
		Cadmium		0.04 mg/L		0.3	
		Mercury		Not Detected		1	
		Arsenic		Not Detected		3	
3	**Aflatoxins**	**Parameters**		**Results**		**Permissible Limits (UPI)**	**Inference**
		B1 G1 B2 G2		0.0002ppm		0.5ppm 0.5ppm 0.1ppm 0.1ppm	Within the permissible limits

4. Conclusion

The TLC/HPTLC fingerprinting studies evaluated with different solvent extracts indicates that the formulation contains many phyto-constituents. Ash values shows the presence of minerals in the drug. The results of WHO parameters viz. microbial contamination, aflatoxins, heavy metals indicates that the drug is free from any toxic substances. From the *in-silico* studies, it has been observed

that the binding energy of phyto-constitutents present in the Unani Joshanda shows negative binding energy with S protein and main protease of SARS-CoV-2. The negative binding energy indicates that the phyto-constituents have the potential to inhibit the S protein and M^{pro} of SAR-CoV-2 and acts as immunity booster. Overall, it is concluded that the study will help the researchers about Unani traditional medicine that are used to combat corona like virus at the time of critical situation like epidemic and pandemics.

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